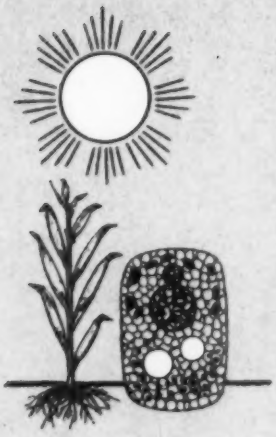
AGRICULTURAL

Science Review

COOPERATIVE STATE RESEARCH SERVICE U.S. DEPARTMENT OF AGRICULTURE VOL. 5 NO. 1

THE INTERNATIONAL
BIOLOGICAL PROGRAM Page 1

FIRST QUARTER 1967



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IBP—New Idiom of Science

As an editor of a science journal, I can easily become excited about the International Biological Program. Why? Because IBP illustrates effective communication among scientists—a concept that science information people have been harping about for years.

Right from its very beginning, IBP represented a purposeful interchange of ideas among scientists. As a matter of fact, the program would never have gained momentum if this interchange hadn't occurred. Both the structure and operational system of IBP are so devised to foster good communication. Moreover, the ultimate success of the program depends largely on how faithfully scientists colloquize.

I suspect these scientists won't fail in their mission. From every corner of the globe, the reports coming in clearly indicate communal thinking among many disciplines. In South Africa, for example, IBP will serve as the theme for the Annual Congress of the South African Association for the Advancement of Science. Here in the United States, the U.S. National Committee is setting up lines of communication to 49,000 biologists and agricultural scientists. In countries such as India, scientists quickly embraced the goals of IBP and national committees lost no time in agreeing on plans of action.

As ultimate users of IBP findings, the public can be assured that the flow of information will permeate to the right places. By its very nature, IBP goals can rightfully be expected to generate action in the science press. As one writer put it: "Man has left his dirty footmarks everywhere, and the time has now come to tidy up."

We can only hope that the scientists are just as excited about IBP as the science writers seem to be. Presumably they are. As evidence, ponder these comments by U.S. National Committee Chairman Roger Revelle: "Our goal should be not to conquer the natural world but to live in harmony with it. To attain this goal, we must learn how to control both the external environment and ourselves. Especially we need to learn how to avoid irreversible change."—W.W.K.

AGRICULTURAL SCIENCE REVIEW is published quarterly by the Cooperative State Research Service, U.S. Department of Agriculture, Washington, D.C. 20250.

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The International Biological Program

On July 1, 1967, begins the operational phase of a program that is perhaps the most ambitious ever undertaken by man for peaceful purposes. With the entire biosphere as its stage, the International Biological Program is geared to answer the increasingly pressing problems of how best to use the Earth's biological resources to support overpopulation by the dominant species—man.

Beginning with this issue, Agricultural Science Review *expects to report regularly on the progress of the program. It seems fitting that the opening report should be written by a man who not only has been active in the preparatory and planning stages of the program, but also holds a strategic post in its functioning here in the United States—Dr. T. C. Byerly, Administrator of USDA's Cooperative State Research Service. He and Stanley Cain (Department of Interior) are vice-chairmen of the U.S. National Committee.*

THE International Biological Program (IBP) is a worldwide plan of research concerned with the biological basis of productivity and human welfare. The parent body of IBP is the International Council of Scientific Unions (ICSU), and at its triennial meeting in London in 1961, the concept of a cooperative biological effort was first conceived. A planning committee was formed in 1962, and a year later the committee made the following significant report:

"It is, therefore, proposed that there be an international program in environmental biology, on the biological bases of productivity and human welfare. The general objectives of the IBP would be to stimulate and coordinate comparative studies, in contrasting environments, of:

"1. Organic production, nutrient cycles, and system regulation on the land, in fresh waters, and in the seas, so that adequate estimates may be made of the potential yield and stability of the new as well as existing resources.

"2. The adaptability of man as a component and manager of these ecosystems."

IBP's aim is to fill in the gaps of knowledge by means of a coordinated, comprehensive approach including sufficient standardization of methods to assure comparable results. Answers will be sought to such questions as the following:

What are the interactions among the many species, especially including man, in aquatic, ter-

restrial, in managed and natural marine, fresh water, and terrestrial ecosystems?

What biological processes determine productivity of ecosystems in tropical, arid, humid, temperate, arctic, high altitude, littoral, pelagic and bathyspheric areas?

How can optimal sustained productivity be increased for man's welfare?

What are the effects of crowding, competition, and nutritive level on population growth, including growth of human populations?

The rapidly increasing human population and the wide extent of malnutrition call for greatly increased food production coupled with efficient management of natural resources. Human activities are creating rapid and comprehensive changes in the environment. Thus, in terms of human welfare, the reason for the IBP lies in its promotion of basic knowledge relevant to the needs for man. It is concentrated on problems the solution of which will benefit from international collaboration.

Organization

IBP is organized internationally as a special committee of the International Council of Scientific Unions (ICSU). The Council consists of unions in each major area of science—biology, physiology, biochemistry, for example. National academies of science in many nations, as in the United States, designate members in the various unions. Thus, ICSU is a nongovernmental organization. The steering committee, which is designated SCIBP, consists of representatives of the Unions of Biological Sciences, Biochemistry, Physiology, Geography, Nutrition, Conservation of Nature and Anthropology and Ethnology; representatives of the ICSU Committees for Oceanographic Research and Arctic Research; of ICSU itself; 10 regional representatives; and the conveners of seven sectional committees. The President of SCIBP is Professor J. G. Baer of Switzerland. Its Scientific Director is E. B. Worthington. The address is 7 Marylebone Road, London, NW 1.

IBP works in close consultation with several of the specialized agencies of the United Nations, particularly the United Nations Educational, Scientific and Cultural Organization (UNESCO), the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Meteorological Organization (WMO), and from these it draws financial or other support.

Subject-Matter Sections

THE International scientific program sponsored by SCIBP is organized into seven sections.

Productivity of Terrestrial Communities—This includes primary productivity through green plants and secondary productivity through animals feeding on plants or on each other. It also includes the breakdown of organic matter.

Production Processes—This is concerned with the utilization of solar energy in photosynthesis and transpiration by plants, and the nitrogen cycle.

Conservation of Terrestrial Communities—Its main task is to provide a scientific basis for the conservation of areas and of species, to assure for present and future needs an adequate supply of natural habitats. These provide unique and rapidly disappearing opportunites for research on biological processes and also have aesthetic values.

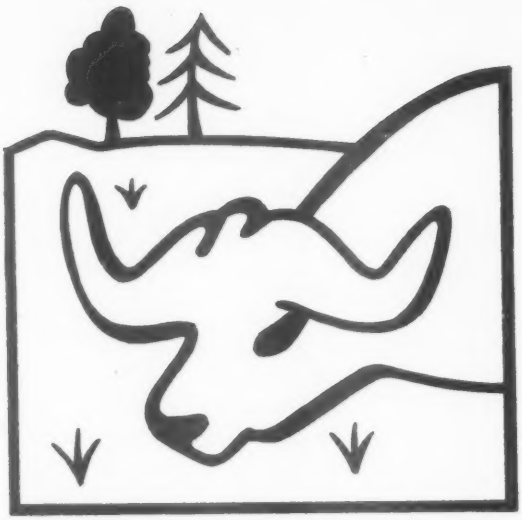
Productivity of Fresh Water Communities—This section has branches concerned with primary and secondary productivity. It includes the conservation of aquatic communities. On the practical side fisheries benefit from an increase of productivity, whereas water supply benefits from the reverse.

Productivity of Marine Communities—This section is tending to concentrate its activities on inshore waters and estuaries for there are several other international organizations dealing with the open oceans. The conservation of marine habitats is included.

Human Adaptability—The subjects covered include genetics, growth and physique, tolerance to heat, cold and high altitudes, working capacity and population dynamics.

Use and Management of Biological Resources—This includes the establishment of plant gene pools, biological control, growth and production of cereals, and the development of new biological resources for man's use.

Each sectional committee met in Paris in April 1966 to plan its own international program and to review relevant proposals from national groups. Since that time, there have been several meetings with SCIBP sectional sponsorship or participation. A meeting on secondary productivity of terrestrial communities was held in Warsaw; a meeting on nitrogen fixation in Moscow; one on the biological



bases of fresh water fish production at Reading, England; one on novel food protein sources in Warsaw; one on biological control of insect pests in Tokyo; and one on gene pools in Rome.

International committees will continue to facilitate cooperation among biologists in participating countries working on problems of mutual interest. They are defining main problem areas in which international cooperation is needed and likely to be productive.

IBP News is the main publication for recording progress. *IBP Handbooks* form a separate series and consist of guides to sectional activities and methods of research. In addition there are publications issued by agencies other than IBP, including books, volumes of symposia results, papers in the technical and semitechnical press. Exchange, storage, and retrieval of data and other information may require designation of institutions willing and able to handle masses of such information.

National Committees

IN the United States and in 37 other participating countries National Committees for the IBP have been formed. Ours is sponsored by the National Academy of Sciences-National Research Council and is administered in the Division of Biology and Agriculture.

The U.S. National Committee (USNC) for the IBP has seven subcommittees paralleling the seven SCIBP subcommittees. We also have subcommittees SB, Systematics and Biogeography, and EP, Environmental Physiology. Roger Revelle, of Harvard, whose enormous abilities are now devoted to human population problems following a distinguished scientific career in oceanography, serves as chairman. Stanley Cain, distinguished ecologist and presently Assistant Secretary of Interior for Fish, Wildlife, and Parks, and I are vice-chairmen of the USNC. Mike DeCarlo and Dick Oliver provide staff assistance to the USNC–IBP.

The USNC is a planning and coordinating body. Its subcommittees hold work conferences to identify research areas relevant to IBP in which U.S. biologists wish to participate and which will benefit from international cooperation. Subcommittees help acquaint biologists with opportunities for participation and, upon request, review proposals for relevance.

Financing IBP

THE major part of the finance for the IBP comes from national sources and is devoted to national research programs. In some cases assistance to national projects may be received through international funds or from foundations. In other cases projects in developing countries may be financed and carried out on a bilateral basis in cooperation with a developed country. The cost of international cooperation, including the central and sectional offices, is relatively small and is provided by participating countries, the specialized agencies of U.N., and by ICSU.

Funds for support of the activities of the U.S. National Committee are provided by Federal research agencies. These agencies have organized an Interdepartmental Coordinating Committee for the IBP under chairmanship of Harvey Carlson of the National Science Foundation. Membership includes the following: Agriculture, Interior, State, Commerce, HEW, Defense, NASA, AEC, and the Smithsonian Institution.

Agencies represented on the Interdepartmental Coordinating Committee have four interests in IBP. First, substantial portions of the intramural and extramural supported research programs funded by these agencies are relevant to IBP and can benefit from international cooperation. Existing projects have been reviewed and relevant por-

tions will be included in the United States program if the agencies and research investigators concerned wish them to be.

Second, opportunity for scientists employed in these agencies to participate in innovative research relevant to the IBP is surely encouraged by agency participation.

Third, participating agencies are, in varying degrees, granting agencies. They will receive without prejudice—and equally without favor with respect to quality—project proposals relevant to the IBP for funding. USNC subcommittees will determine relevance. They can help, as all competent scientists must help, in advice on form and procedure. But the granting agencies are solely responsible for evaluation of merit and allocation of scarce research funds. Project and program proposals relevant to IBP must meet these quality standards.

Fourth, Interdepartmental Coordinating Committee will review, and—within agency policy, means, and evaluation—fund the requirements of the U.S. National Committee.

Significance to Agriculture

OBVIOUSLY, there are many areas in the IBP of particular relevance to the agricultural disciplines. A survey of the seven subject-matter sections reveals the broad scope. Inherent in the IBP is the necessity for imaginative research designed to maintain a self-sustaining chain of food production. To do so requires a systematic study of the natural resources that still exist. An accurate count of animal and plant species and their distribution, an examination of the interaction of communities upon each other, what sustains them and whether they have protective or adaptation mechanisms—all these and more are relevant to the agricultural disciplines. The problems are global. It is therefore axiomatic to tackle them internationally.

Of all the niches, all the species—is man egocentrically dominant? Or is he not? Will he finally succumb to the mess he has permitted to develop? Is he ignorant of the final constraints of the ecosystem on his own well being? Probably not. Man is highly adaptive; he will survive in any foreseeable ecosystem, rationalize it, and even believe that he likes it and wants it that way. A few grumbletonians will shout doom and gloom—a nuisance to their fellows and an annoyance to themselves.

T. C. BYERLY, *Administrator,*
Cooperative State Research Service,
U.S. Department of Agriculture.

How To Participate in IBP

If you are interested in participating in IBP, first examine its theme and defined goals. Then, if your research competencies seem to fit the program, proceed according to the following plan:

Draft your proposal in the usual project form and submit it to your own or a granting agency. Send a copy to the Executive Secretary, USNC, International Biological Program, Division of Biology and Agriculture, National Academy of Sciences-National Research Council, 2101 Constitution Avenue, Washington, D.C. 20418.

If you need assistance in preparing a proposal, write to the appropriate subcommittee chairman or to the IBP headquarters.

Progress in Bloat Prevention

ERLE E. BARTLEY

BLOAT of cattle and sheep is estimated to cause a $100 million annual loss in the United States. Although large numbers of animals die of bloat, probably the greatest economic losses from bloat occur from decreased animal usefulness and restricted use of high-yielding legume pastures because of the fear of bloat.

A number of comprehensive reviews have been written about bloat, and the volume of research literature on the subject is quite extensive. This article makes no attempt to supplement either of such categories, but, rather, presents a factual summary consisting chiefly of the experiences of Kansas research workers.

Bloat does not seem to be the result of a simple mechanism; instead, it is a complex one in which several factors interact simultaneously. This complex balance of factors undoubtedly accounts for the unpredictability of bloat.

Bloat usually occurs when cattle graze legumes or are fed rations containing a high proportion of concentrates with protein supplements as are fed to fattening cattle in feedlots. Feedlot bloat and bloat resulting from eating legumes are similar in that excessive foaming of ruminal digesta occurs in both. When cattle are fed bloat-provoking rations, the consistency of the ruminal digesta increases, and gas—a normal product of rumen fermentation—becomes trapped in the digesta to form a stable foam. Evidence has been obtained that foam can inhibit eructation, or belching (*10*).[1]

[1] Italic numbers in parentheses refer to Literature Cited, p. 12.

When frothing agents are absent, excessive gas production in the rumen is usually no problem, since the cow can void gas by eructation. However, when frothing compounds are present, excessive gas production—particularly that occurring from readily metabolizable carbohydrates in very young legume pastures or in grain in feedlot rations—will provoke bloat.

Some animals will bloat on any ration and are commonly referred to as chronic bloaters. Usually, the rumen digesta in these animals does not foam extensively, and abdominal distention is due to free gas pressure which results because the animal is unable to eructate fermentation gases. Eructation is inhibited possibly because of anatomical defects. Although chronic bloat sometimes can be controlled effectively by surgical establishment of small ruminal fistulae, such measures are not always practical or economical. Ruminal introduction of a stomach tube or hypodermic needle will deflate the rumen and provide temporary relief.

Plant Factors

IT is commonly recognized that plants with a high nitrogen content such as legumes are more bloat provocative than nonlegumes. There is ample evidence that cytoplasmic proteins are of major importance in producing foam (*6*). Kansas work has shown a decrease in bloat incidence with increasing maturity and decreasing protein content of alfalfa plants (*16*). Analyses of alfalfa bloat foams indicated that the foaming constituent is primarily proteinaceous (*2*). However, the quantity of protein per se apparently is not necessarily the factor that determines whether a plant is bloat provocative. For example, birdsfoot trefoil, a leguminous plant high in protein, is seldom bloat provocative. Recent Iowa work has revealed a relationship between foaming potential and soluble protein content of the plant (*22*). Alfalfa extracts gave maximum foam stability and maximum soluble protein content during prebloom growth, whereas birdsfoot trefoil was relatively low in soluble protein. McArthur and Miltimore (*14*) have isolated a foam-stabilizing protein from alfalfa whose physical and chemical properties make it an ideal bloat-promoting agent. Soluble protein content may be a good indicator of a plant's bloat-provoking potential.

Although proteins appear to be the most important plant factors associated with bloat, numerous other plant components have been studied (*6, 10*). Saponins produce extremely stable foams and may be related to bloat. Plant pectins and particulate matter may act as foam stabilizers once foams are formed in the rumen.

Plant lipids and tannins are possible foam inhibitors. Many workers (*6*) have partially succeeded in preventing bloat by using various plant oils and animal fats. Plants with a high tannin content have been found not to be bloat provoking (*12*).

Minerals may also be involved in the production of bloat (*6*). Adequate mineral supplementation is necessary for abundant and rapid plant growth. Some of the polyvalent minerals have definite effects on colloidal behavior and may stabilize foam.

In general, any plant constituent that decreases with increased plant maturity correlates well with the incidence of bloat. Moreover, any constituent whose content increases with maturity correlates well with the apparent absence of bloat. Also, any condition which inhibits rapid growth is associated with the absence of bloat. This fact confounds the problem because it is difficult to sort out the constituents that are responsible for foam formation and stabilization and those that inhibit foam formation.

Animal Factors

A PLANT containing foam-forming substances does not insure that it is bloat-promoting to all animals. This situation is obvious when only some animals in a herd bloat when grazing a bloat-provocative pasture. An inherent susceptibility to bloat among animals is amply demonstrated by research with identical twins. Great differences in bloat susceptibility have been noted among twin sets, but similar susceptibility shows up in both members of a twin set.

Also, if foam-forming compounds in green alfalfa were the only factors involved in bloat production, bloat would be produced on hay as easily as on succulent alfalfa pasture, because alfalfa hay contains the same foam-producing compounds in nearly the same concentrations as green forage. Very stable foams can be made from the hays by grinding them and shaking them with water. This phenom-

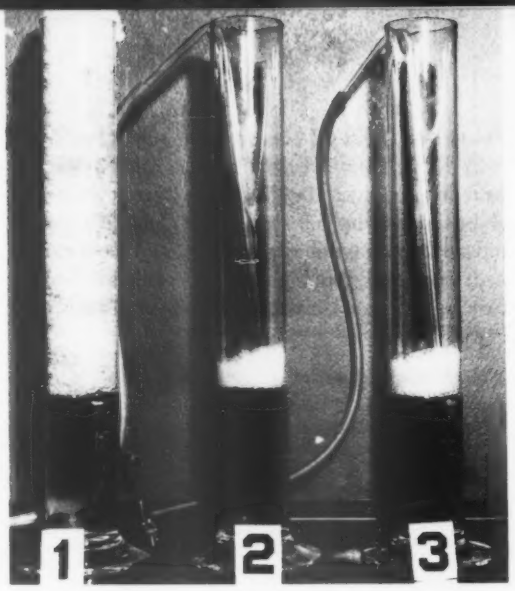


FIGURE 1.—Antifoaming effect of bovine saliva (cylinder 2) and mucin (cylinder 3) on alfalfa saponin foam (cylinder 1).

enon was somewhat of a stumbling block to a satisfactory explanation of the bloat complex. Bloat must result from the *presence* of foam-promoting compounds coupled with the *absence* of an antifoaming agent of animal origin.

Results of our studies suggest the possibility that the antifoaming agent for alfalfa hay is saliva. Studies reported in 1961 had demonstrated that saliva, or more specifically the mucin in saliva, is an effective antifoaming agent when associated with foaming rumen digesta (*23*). Adding saliva to incubated frothing rumen contents (table 1) permitted greater quantities of gas to escape than when no extra saliva was added. Adding saliva to an alfalfa saponin solution (fig. 1) almost completely prevented formation of foam when the solution was aerated (*5*). Cows fed alfalfa hay secreted approximately twice as much saliva per feeding as when fed freshly cut alfalfa (*17*). A total daily secretion of 117 to 183 kg. of saliva per animal was estimated when hay was fed. Salivary secretion increases as water content of feed decreases (table 2, fig. 2). Significantly, it was observed that nonbloaters secrete greater quantities of saliva than do bloaters—a logical explanation for hereditary differences among animals in susceptibility to bloat.

TABLE 1.—*Effect of saliva and linseed meal mucin solution on release of gas from frothing rumen contents and the effect of saliva on the production of gas by nonfrothing rumen contents*

Treatment	Number of trials	Gas release from 200 gm. rumen contents (ml.)			
		15 minutes	30 minutes	45 minutes	60 minutes
Frothing Rumen Contents					
Control	12	51	93	129	150
20 ml. mucin	12	[1] 58	[1] 105	[1] 147	[1] 175
20 ml. saliva	12	[1] 62	[1] 107	[1] 146	[1] 171
80 ml. mucin	12	[1] 68	[1] 124	[1] 174	[1] 197
80 ml. saliva	12	[1] 71	[1] 129	[1] 177	[1] 202
Nonfrothing Rumen Contents					
Control	4	20	32	65	115
80 ml. saliva	4	19	30	64	121

[1] Significant at the 1-percent level.

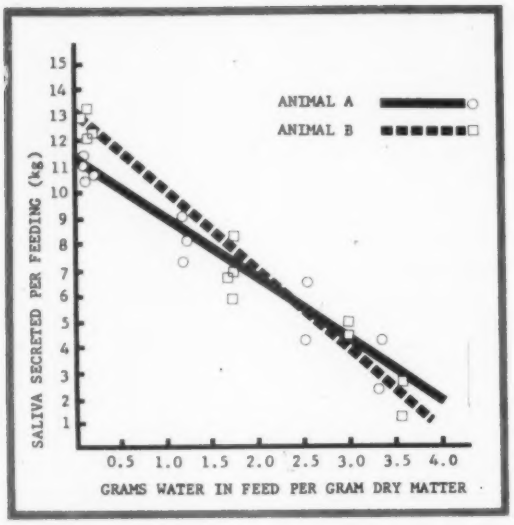


FIGURE 2.—Effect on the estimated total saliva production per feeding of adding water to alfalfa hay.

It has been postulated that bloat results when feeds containing foaming constituents fail to induce sufficient salivary secretion. This would explain why cows on young, succulent legumes are more susceptible to bloat than those feeding on more mature legumes or hay. Also, damp or wet legumes are more bloat provoking than the same plants on a dry day, possibly because of reduced salivary flow. Young, succulent grasses usually do not induce bloat, even though they do not provoke much salivary secretion, because they lack the chemical components that augment foaming. Occasionally bloat is reported when cattle graze young, succulent grasses such as wheat. Such grasses provoke bloat because they usually contain large quantities of foam-forming proteins.

Microbiologic Factors

DURING studies on the role of saliva in bloat, the question was raised whether bacteria capable of degrading mucin exist in the rumen. Organisms were isolated from the rumen and tested for mucinolytic activity. Several bacteria capable of breaking down salivary mucin and utilizing free neuraminic acid were found (*8*). When cultures of the organisms were introduced into rumens of fistulated cows grazing a mature non-bloat-provoking pasture, or consuming sub-bloat-provoking feedlot rations, bloat resulted in the majority of cases (table 3). Rumen digesta of animals fed freshly cut alfalfa or a feedlot bloat ration contained more aerobic and anaerobic mucinolytic bacteria (particularly in bloated animals) then did those fed alfalfa hay (*20*). Also, rumen fluid of bloated animals either grazing alfalfa or fed alfalfa hay or both possessed greater mucinolytic activity than that of nonbloated animals on the same feeds.

TABLE 2.—*Saliva secreted while eating lush or mature pasture*

Type of pasture	Water content of alfalfa per kg. dry matter	Twin pair	Saliva (kg.) secreted per kg. dry matter consumed
	Kg.		*Kg.*
Lush	6. 32	04	[1] 1. 61±0. 73
		05	2. 08±0. 68
		20	1. 13±0. 17
		21	1. 30±0. 03
			1. 53 (Mean)
Mature	4. 51	04	3. 66±0. 64
		05	3. 53±0. 60
		20	2. 49±0. 05
		21	3. 50±0. 30
Mean			3. 30 (Mean)

[1] Standard deviation.

TABLE 3.—*Development of feedlot bloat in cattle inoculated with a mucinolytic rumen organism*

Number of twin pairs	Treatment	Average maximal bloat index observed [1]					
		Day before inoculation	Days after inoculation				
			1	2	3	4	5
3	Inoculated	0.3	3.4	3.2	3.2	2.6	2.7
3	Control	.8	.9	.8	.8	.6	.8

[1] Each value is an average of 3 replicates. Bloat index based on rating from 0 (no bloat) to 5 (severe bloat).

Hence, it was postulated that mucin in normal salivary secretion, if sufficient in quantity, prevents bloat. If salivation is reduced during consumption of bloat-provoking diets, or if mucin is destroyed by excessive concentration of mucinolytic flora, bloat may result.

Micro-organisms may be related to bloat in other ways. Slime produced by rumen micro-organisms may contribute frothing factors to legume or feedlot bloat. In Kansas studies (*3*), rumens of fistulated identical twin cows were emptied, washed with water, and replaced with (a) 4 liters of natural rumen fluid, or (b) 4 liters of autoclaved rumen fluid. Both kinds of rumen fluid were from bloating animals. After the rumen fluid was added, both groups of identical-twin cows were then pastured on succulent alfalfa. Cows given the natural fluid bloated the second day. Cows given the autoclaved fluid bloated the fourth day, but had consumed almost optimum amounts of bloat-producing feed the second and third days. Those results—plus those from a bacteriologic study—suggest that bloat does not result from a simple physical breakdown of feed but may depend on establishing a certain concentration of micro-organisms. Control of bloat by antibiotics further suggests an association between micro-organisms and bloat. Antibiotics may prevent bloat by (a) destroying mucinolytic bacteria, (b) slowing down gas production, and (c) by inhibiting bacterial processes involved in forming rumen foam.

Prevention of Bloat

AS stated earlier, bloat is a complex condition in which at least three factors interact simultaneously—the plant, the animal, and the rumen microbial population. Eliminating the *effect* of any one of the three factors would be difficult and would probably lead to inefficient animal production. Therefore, since bloat is essentially a problem of foam developing in the rumen, it was reasoned that a simple approach to bloat control might be through physical control of foam with antifoaming agents. Although several antifoaming agents such as silicones, detergents, vegetable oils, tallows, and liquid paraffins have been used with some success, even the most effective of them have not proved entirely satisfactory in their natural form.

Nevertheless, screening tests at Kansas State University continued during the past 7 years in an attempt to discover a practicable antifoaming agent to control alfalfa bloat. All compounds were subjected to seven critical criteria. One administration of an antifoaming agent must:

- Effectively prevent bloat at least 12 hours.
- Act rapidly (within 10 minutes).
- Be palatable.
- Cause no deleterious effects on health, reproduction, rumen function, feed intake, or quality or quantity of milk.
- Not be eliminated in milk.
- Not be found in body tissues 5 days after administration.
- Be economical.

To obtain such a compound it was necessary to screen numerous antifoaming agents both singly and in combination. Identical-twin cattle that were both bloat-susceptible and rumen-fistulated proved to be extremely effective screening tools. It is doubtful that this work would have been completed in 7 years if identical twins with known bloat histories had not been used.

After screening numerous compounds, it was apparent that many would fulfill some but not all of the established criteria. Some were very effective but only for short duration; others had to be rejected because they lacked "staying power." Several that were effective when given in large quantities proved to be impractical from a standpoint of method of administration and cost. Several were discarded because they were either toxic or unpalatable. A few excellent antiforming agents failed because they did not disperse readily in the rumen digesta. The silicones were in this category. Their effectiveness was enhanced greatly by improving their dispersibility; however, the cost of such modification prohibited their use.

Over the years, the acquired knowledge on the behavior of many of these compounds in the rumen and a knowledge of the physical and chemical characteristics of the rumen during bloat enabled us to develop specifications for an effective bloat prevention agent. The polyoxypropylene polyoxyethylene block polymers appeared to fit many of the requirements.

By chemical alterations it was possible to change physical properties of dispersibility, wetting, solubility, etc., and by trial and error it was possible to determine what changes were needed to construct an efficient bloat-preventive agent. Several compounds were synthesized. After extensive testing, poloxalene [2] emerged as the product most closely meeting the established criteria.

In 4 trials using rumen-fistulated, identical-twin dry cows, levels of 5 to 20 gm. of poloxalene were administered once daily before the animals were fed green alfalfa (*1*). The degree of bloat was rated by a score ranging from 0 (no bloat) to 5 (severe bloat) (table 4). Sets of fistulated identical twins especially selected for bloat susceptibility provided a precise and rapid method to screen antifoaming agents. (Effects of the antifoaming agent on froth development can be observed in animals known to produce froth with great regularity.) The goal of this and other investigations had been to find an agent that would prevent uncomplicated legume bloat 100 percent of the time. A definite bloat condition (bloat score from 3 to 5) in an experimentally treated animal on only one occasion would indicate a possibility of many animals bloating under field conditions. Results (table 4) showed that no animal treated with poloxalene had a bloat score above 1.5 during 12 hours following treatment, indicating that poloxalene approached the desired goal.

In field trials (*4*) half the cows of each of four Kansas dairy herds served as controls, while half were fed poloxalene at 5 or 10 gm. per cow twice daily. All were given succulent alfalfa. Controls were reversed after 7 to 10 days. In three herds, prevention of bloat by poloxalene approached 100 percent; in the fourth, a few large Holstein-Friesians fed poloxalene bloated at a level of 20 gm. per day, but bloating ceased when the level was doubled. In a second experiment, cows in five Kansas dairy herds were given poloxalene by various methods for 29 to 71 days. As in the first experiment, bloat did not occur when the level of poloxalene was adequate.

In a 113-day trial completed recently at Iowa State University (*4*), half of 36 steers were fed 10 gm. of poloxalene daily. All 36 were fed high quality, freshly cut alfalfa. Bloat was high in those that received no poloxalene; only a few fed poloxalene bloated. In each case the animal had consumed less than half the poloxalene offered.

Before poloxalene could be authorized for farm use, it was necessary to determine if it was eliminated in milk or deposited in body tissues. That information was obtained by administering labeled poloxalene (C^{14}) to a lactating cow (*18*). C^{14} activity did not appear in milk or in body tissues 9 days following administration of the drug. Since 94.3 percent of the C^{14} activity administered was recovered in feces and 4 percent in urine, it was concluded that the surfactant is poorly absorbed from the digestive tract. Chemical analytical methods to detect the drug were developed by Meyer *et al.* (*19*) at Kansas. Results from six studies with animals fed recommended levels of poloxalene revealed no detectable residues in either milk or meat. Only trace quantities were detected after feeding four to five times the recommended dosages. Rats were used to determine toxicity of poloxalene. Huge dosages showed no toxicity (*13*).

Two experiments were conducted to determine the effect of poloxalene on lactating dairy cows (*9*). In the first, six cows were fed varying level of poloxalene (10, 20, or 40 gm./cow/day) for 12 weeks,

[2] Smith Kline and French Laboratories, No. 18667, Philadelphia, Pa.

and six cows served as controls. The surfactant had no deleterious effect on milk production, milk fat test, body weight, feed consumption, conception rate, or animal health. In a second experiment, two sets of fistulated identical twins were used to determine the surfactant's effect on rumen fermentation. Poloxalene had no effect on rumen ammonia concentration, rumen pH, or rumen lactic and volatile fatty acid concentration. Inoculums from surfactant-fed and control animals digested similar quantities of cellulose *in vitro*. It was concluded that poloxalene does not affect rumen fermentation.

Poloxalene was approved for use as a top dressing on feed by the Food and Drug Administration in March 1966. It may be sprinkled over and stirred into grain and fed to cattle before they are given succulent legumes. Recently several studies have been conducted to test the bloat-preventive effect of poloxalene incorporated in 33 lb. molasses-salt blocks. These blocks contain 30 gm. poloxalene per pound and cattle on pasture will consume approximately 0.5 to 1.0 lb. of block daily.

In studies conducted at Mississippi (*7*) and Louisiana (*11*) the poloxalene-molasses-salt blocks effectively reduced severity and incidence of bloat.

TABLE 4.—*Effect of poloxalene on bloat in identical twin cows fed green alfalfa*

Trial No.	Number of days	Number of animals	Treatment [1]	Average bloat index		Range in bloat scores	
				Morning	Afternoon	Morning	Afternoon
I	2	2	Control	2.6	3.1	1.5–3.0	2.5–4.0
	2	2	20 gm. poloxalene via rumen.	0	.3	0	0–1.0
II	3	4	Control (pretreatment).	2.0	1.4	0–3.5	0–3.5
	3	4	20 gm. poloxalene via rumen.	[2] 0	[2] .1	0	0–1.0
	3	4	Control (posttreatment).	[2] .3	[2] .3	0–2.0	0–2.0
III	3	.....	Control (pretreatment).	2.2	1.5	0–3.0	0–3.0
	3	4	10 gm. poloxalene in grain.	[2] .3	[2] .5	0–1.0	0–1.5
	3	4	Control (posttreatment).	2.1	2.3	.5–3.5	.5–3.5
	2	4	20 gm. poloxalene in grain.	[2] .4	[2] .4	0–1.0	0–1.0
	2	4	Control (posttreatment).	2.0	1.0	.5–3.0	0–3.0
IV	1	4	Control (pretreatment).	3.1	2.5	3.0–3.5	2.0–3.0
	3	4	5 gm. poloxalene in grain.	[2] 0	[2] .6	0	.5–1.0
	3	4	Control (posttreatment).	2.0	1.7	.5–3.5	0–3.0

[1] Per 450 kg. body weight.
[2] Significantly different from pretreatment control ($P<.01$).

In a study recently completed in Kansas (*21*) cattle receiving molasses-salt blocks without poloxalene had 54 severe cases of bloat requiring treatment and 3 head died of bloat. Paired animals receiving molasses-salt blocks containing poloxalene were relatively free of bloat. None bloated severely and none required treatment. The poloxalene-molasses-salt blocks appear to be an effective method of administering poloxalene, especially for cattle not normally receiving a grain carrier for poloxalene. Studies at all three locations emphasized the need for careful management of the block to insure adequate intake. It was necessary to place the blocks in areas where cattle congregate when not grazing. Efficacy data have been submitted to the Food and Drug Administration and it is hoped that approval for the block dosage form may be granted in the near future.

Conclusions

BLOAT control may be the result of a physical effect of poloxalene on foam formation. Preliminary results from Iowa and Kansas State Universities show surface tension of rumen fluid from poloxalene-fed animals to be about 10 dynes/cm. lower than fluid from controls. Results of the isotope study suggested that poloxalene is not readily degradable in the rumen. This appears to explain the fact that treatment levels of 5 to 20 gm. of poloxalene administered each morning completely prevented abdominal distention for at least 12 hours in animals fed green alfalfa. Additional studies on the mode of action of poloxalene in controlling bloat are in progress in Iowa and Kansas. Most of the work with poloxalene has been with legume bloat. The effectiveness of poloxalene in controlling feedlot bloat has yet not been adequately tested.

As noted earlier, chronic bloaters with low foam production do not respond to treatment. Further studies on the mechanism of action of poloxalene may provide further insights into mechanisms which are common to all three forms of bloat, and thus lead the way to discovery of even more effective chemical control of certain of these forms.

Despite favorable results with the use of poloxalene, the problem of bloat is by no means completely solved. Our studies clearly indicate that further research is needed to determine the cause of the problem. Some change in the ruminal flora may be involved. We need to know how the flora differ between bloating and normal animals. Certainly, it seems apparent that—since management and feeding practices for dairy cattle are changing—additional research on feedlot bloat is imperative.

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MYCORRHIZAE: Indispensable Invasions by Fungi

EDWARD HACSKAYLO

IN an earlier issue of this journal, Waksman (*19*)[1], aptly stated, "The soil is not a mass of rocks and residues; it is not a dead organic-inorganic system, but a living system teeming with numerous forms of life." Of the several forms of life he mentioned, roots of higher plants and certain fungi are involved in one of the most intriguing of natural phenomena. Specific fungi grow upon and vigorously invade portions of root systems that are primarily responsible for nutrient absorption by higher plants. In 1885, Frank (*4*), a German forester, coined the term "mycorrhiza," meaning fungus-root, to designate these particular invasions of roots by fungi. Without mycorrhizae, many plants, including our most important timber species, could not survive in the dynamic, fiercely competitive biological communities that abound in natural soil habitats.

On the basis of the interrelation of threadlike fungus hyphae and the root cells, mycorrhizae are divided into two general classes—ectotrophic and endotrophic. The kind is usually specific for the genus of higher plant on which it occurs, and mycorrhizae have been found on most genera of seed plants that have been examined. Ectotrophic mycorrhizae are common on the pine (*Pinaceae*) family among gymnosperms and on the birch (*Betulaceae*), beech and oak (*Fagaceae*) and a few other families among angiosperms.

Endotrophic mycorrhizae are on the roots of most shrubs, and certain trees such as maple, yellow poplar, sweet gum, redwood, and apple among others. In fact, they occur on most genera of seed plants that do not possess ectotrophic mycorrhizae.

Ectotrophic mycorrhizae are found on some of the most important tree species. The associated fungi are more readily identified and cultured than endotrophic mycorrhizal fungi, and for this reason they have been studied much more intensively than the endotrophic. Our investigations, too, have dealt primarily with ectotrophic associations. This discussion will place major emphasis on certain aspects of ectotrophic mycorrhizal associations, after brief comments on endotrophic mycorrhizae.

[1] Italic numbers in parentheses refer to Literature Cited, p. 20.

ENDOTROPHIC MYCORRHIZAE

THE invading organisms in endotrophic mycorrhizae are primarily inconspicuous phycomycetous fungi that produce subterranean, nearly microscopic, fruiting bodies. Endotrophic fungi are present on surfaces of the mycorrhizal rootlets as individual threads or loose hyphal wefts. The fungi secrete cellulolytic enzymes that dissolve a minute portion of the cell wall, thus allowing the hyphae to penetrate root hairs and other epidermal cells. Penetration sometimes is limited to the epidermis, but frequently hyphae grow into the cortex cells. Hyphae appear within the cells as coils (fig. 1), swellings, or minute branches where their digestion by the host cells is common. The meristematic tip and vascular region are not invaded. The gross morphology of the roots may remain essentially unchanged or may become beaded (fig. 2). Beading is caused by periods of arrested growth followed by periods of active growth as the result of changes in the micro environment. Endotrophic mycorrhizae usually are darker in color than nonmycorrhizal roots; however, they develop no pathological symptoms.

Orchid endophytes are generally classed as endotrophic mycorrhizal fungi. In their evolutionary development these fungi are among the more advanced within the Basidiomycetes. The orchid embryo, except under special artificial nutritional circumstances, requires penetration by a specific fungus for completion of its development; otherwise seed germination does not occur. Oddly enough, some of the orchid fungi, such as *Rhizoctonia* spp. and *Armillaria mellea* are serious pathogens on other hosts.

FIGURE 1.—Section through an endotrophic mycorrhizae of red maple.

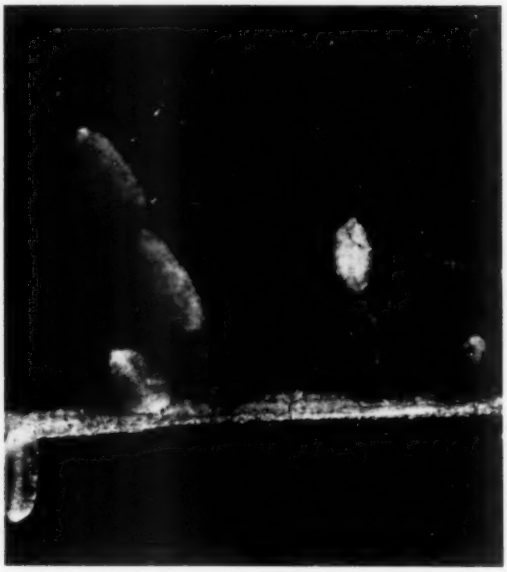

FIGURE 2.—Endotrophic mycorrhizae of red maple.

With the exception of orchid fungi, the significance in the physiology of host plant in endotrophic mycorrhizal associations has been very obscure. One of the primary reasons for this is that endotrophic fungi are very difficult to isolate and culture. Mosse (*14*), in England, found that by using spores of *Endogone* she was able to inoculate rooted apple cuttings and produce a stimulating effect to the inoculated cuttings. Others have reported marked stimulation in the growth of yellow poplar, *Griselinia,* and a few other species. Gerdeman (*5*), even demonstrated marked stimulation in the growth of maize by inoculating with spores of *Endogone.* Thus, we have entered a new phase in the studies on endotrophic mycorrhizae. Daft and Nicolson (*3*), in Scotland, recently showed that endotrophic mycorrhizal fungi caused a marked increase in the uptake of phosphorus in maize and tomato compared to nonmycorrhizal plants when phosphate availability was low. There is the likelihood that the uptake of other inorganic nutrients is increased by the hyphae attached to the roots that branch out into soil adjacent to the mycorrhiza. It is possible that digestion of hyphae by root cells also releases their nutritive contents to the host.

A most intriguing discovery was made by Mosse (*15*) when she found that working in axenic culture with clover seedlings inoculated with disin-

fected spores of *Endogone,* infection by the mycorrhizal fungus was very slight unless a third organism, a species of *Pseudomonas* or other species of bacteria, was present in the rhizosphere. The complexity involved in establishment of endotrophic mycorrhizae is still not well understood. It seems likely, however, that the bacteria are contributing enzymatically in the establishment of the mycorrhiza. The next few years should provide some very interesting data on this long avoided research subject.

ECTOTROPHIC-MYCORRHIZAE

THE occurrence of ectotrophic mycorrhizae is widespread, particularly in temperate regions. Mycorrhizal association is usually confined to those roots in the top few inches of soil. The smallest of the secondary roots are invaded by fungi during periods of active root growth. With few exceptions, the fungi involved produce mushrooms as fruiting bodies. Attachment of hyphae to the roots apparently is requisite to fruiting under natural conditions, and as a result mushroom epicures are well aware that certain species of mushrooms are found only under certain trees.

The sequence of events in ectotrophic mycorrhizal formation follows a rather specific pattern. The fungus gains contact with a susceptible root from germinating spores, or hyphae from other propagules in the rhizosphere. Stimulated into rapid growth by root exudates, the hyphae envelop the entire root tip as a dense sheath. As the root cells divide and elongate, growth-regulatory compounds secreted by the fungi cause marked changes in the morphological development of the root as compared to nonmycorrhizal roots. Root hairs do not develop. The mycorrhiza is shorter than the nonmycorrhizal root, and sometimes, as in pine, it is dichotomously branched once or many times (fig. 3). Slankis (*18*) in a series of classical experiments, correlated these effects with auxin production by mycorrhiza fungi. By secreting enzymes, the fungus is able to penetrate the root but is restricted to the intercellular region of the cortex (fig. 4). The meristematic tip and the vascular cylinder are not invaded. Consequently, a very organized and morphologically distinct "organ" develops. The fungus and root cells retain their vital characteristics and show no pathological symptoms.

Ectendotrophic Mycorrhizae

IN addition to ectotrophic and endotrophic mycorrhizae, ectotrophic tree roots, primarily in nurseries, develop the typical intercellular organization of ectotrophic mycorrhizae plus intracellular penetration by hyphae. These are ectendotrophic mycorrhizae and were originally thought to represent a transitional stage between the ectotrophic and the endotrophic type. However, Mikola (*13*), in Finland, has presented strong evidence that ascribes the formation of at least some ectendotrophic mycorrhizae to an isolated but as yet unidentified fungus. If transplanted on roots of seedlings from a nursery to a forest soil, the fungus apparently ceases to develop and gives way to ectotrophic mycorrhizal fungi. Because of the prevalence of ectendotrophic mycorrhizae in forest seedling nurseries, these findings deserve more intensive physiological investigation.

Physiological Significance

IT is impossible to pinpoint the first person who saw a mycorrhiza and recognized it as being comprised of two organisms. In the latter part of the 19th century, however, European investigators began to speculate on the physiological significance of mycorrhizae. All of their conclusions were based primarily on observations of the morphology of naturally occurring structures.

Since a compact fungus mantle isolates the root tissue from the adjacent soil, Frank (*4*), in 1885, thought that a symbiotic relationship must exist. He theorized that water and organic nutrients were absorbed through the fungus, then translocated into the tree. Hartig (*7*) shortly thereafter expressed doubt that mycorrhizal fungi were anything but harmless parasites. Considerable controversy followed, and the latter idea had support off and on for nearly 50 years in Europe and the United States.

In 1917, Professor Elias Melin, at Uppsala, Sweden, initiated new and significant approaches to studies on mycorrhizae of pine, spruce, and other Scandinavian tree species. He observed that in drained peat bogs only those trees whose roots become mycorrhizal grew normally (*11*). Thereafter he directed his efforts towards research on the physiology of tree mycorrhizae under carefully controlled laboratory conditions. He eliminated the complexity of the soil microbiota by developing

techniques for growing the fungi and host plants in axenic culture. By combining cultures of the two, he demonstrated clearly which fungi were responsible for mycorrhizal formation.

Early in his investigations, Melin believed that ectotrophic mycorrhizae were essential in the nutrition of trees. He supported previous concepts of inorganic nutrient uptake, but also hypothesized that organic nitrogen compounds might be absorbed by the hyphae and translocated into the root tissues. During the 1930's, Hatch (*8*) theorized that mycorrhizae were considerably more effective in nutrient uptake than nonmycorrhizal roots because of the increased surface area provided through root proliferation and of the large absorbing surfaces of hyphae in the mantle and surrounding soil. His data showed that mycorrhizae were most effective in soils moderately deficient in nitrogen, phosphorus, and potassium. By analyzing tissues of mycorrhizal versus nonmycorrhizal seedlings Hatch found increases of 86 percent nitrogen, 234 percent phosphorus, and 75 percent potassium over the nonmycorrhizal seedlings.

FIGURE 3.—Ectotrophic mycorrhizae of Virginia pine.

Others in the United States and elsewhere accumulated evidence supporting these findings. In 1949, Kramer and Wilbur (*9*) observed that mycorrhizae of pine accumulated radioactive phosphorus to a greater degree than nonmycorrhizal pine roots. In 1950, and for several succeeding years, Melin and his coworkers in a series of experiments clearly demonstrated in axenic culture that mycorrhizal fungi transported radioactive inorganic and organic nitrogen, phosphorus, calcium, and sodium from the substrate into roots of Scotch pine. The efficiency of the mycorrhizal system was far greater in each case than in the nonmycorrhizal root. Radioactive isotopes transported into the root tissues were readily translocated into other parts of the seedlings. Thus, after many years of speculation and circumstantial evidence, Melin proved conclusively that ectotrophic mycorrhizal fungi play a very active role in nutrient uptake by trees.

Erik Björkman (*1*), a student of Melin's, carried out a series of experiments on the effects of light intensity on the development of mycorrhizae on Scotch pine. With diminishing intensities of light, there was a proportionate decrease in the numbers of mycorrhizae formed. At about 12 percent of full

FIGURE 4.—Part of a transverse section of an ectotrophic mycorrhizae of Virginia pine.

sunlight and lower, mycorrhizae ceased to form. Björkman concluded from chemical analyses of roots that unless sufficient quantities of available carbohydrates were present in the root systems, mycorrhizal fungi would not penetrate the roots. He further demonstrated this by cutting off the flow of photosynthate from the shoots to the roots by strangulation with a wire and found again that mycorrhizae did not form even though appropriate fungi were present in the substrate. His data provided convincing evidence that mycorrhizal fungi were receiving carbon compounds from the host plant. Later, Melin and Nilsson (*12*) conclusively demonstrated this by subjecting shoots of Scotch pine to carbon dioxide with the carbon atom labelled. Within a matter of hours they detected the radioactive carbon in the mycelium of an associated mycorrhizal fungus.

Recently we (*6*) found that fruiting by the mycorhizal fungus, *Thelephora terrestris,* is dependent upon the photosynthetic capabilities of pine. If light is excluded from the needles, formation of the fruiting body immediately ceases. When the light is resumed, the fruiting body continues to develop.

Over the past 15 years, J. L. Harley and his students and coworkers in England have been very active in elucidating many of the biochemical and biophysical reactions that take place in ectotrophic mycorrhizae. Working primarily with mycorrhizae of beech, they have concentrated on the uptake, accumulation, and transport of phosphorus from the substrate into the host tissues and pathways of carbon metabolism in mycorrhizae. They have employed radiological techniques to great advantage in exploring these fundamental metabolic processes.

Monotropa

ONE of the most interesting mycorrhizal associations that has attracted attention for over a century is that of *Monotropa,* the species that we know commonly as Indian pipe. *Monotropa* is a seed plant belonging to the Heath family, but contains no chlorophyll. The roots are so profusely mycorrhizal that the entire root system is isolated from the soil by a dense mycelial mantle. The associated fungi are species that also form ectotrophic mycorrhizae on certain trees. The source of carbohydrates within the *Monotropa* was thought to be from litter decomposition by mycorrhizal fungi or other organisms in the immediate vicinity of the mycorrhizae. Thus it was concluded that the mycorrhizal fungi could transport the carbohydrates to the host, and that was the source of carbon compounds used in its metabolic processes.

In 1960, Björkman (*2*) injected the trunks of spruce trees with radioactive carbon and phosphorus compounds, then analyzed tissues of *Monotropa* plants growing beneath these trees. Radioactivity, detected in the fungus mycelium and the tissues of the *Monotropa,* indicated that a mycelial bridge provides a pipeline for transport of carbon and other compounds from roots of spruce trees into the roots of *Monotropa.*

Although most of the other research studies cited have been directed toward explaining the physiological significance of ectotrophic mycorrhizae, many other facets of mycorrhizae have been studied. Morphological descriptions have identified the types of mycorrhizae and their individual characteristics on particular host species. Field observations followed by laboratory syntheses have positively identified many mycorrhizal fungi. Ecological observations and experiments have attempted to define factors affecting range and distribution of many host and fungus species. Data are being compiled on several fronts that will continue to bring together a more complete story on ectotrophic mycorrhizae. As a consequence we can begin to approach the practical significance of mycorrhizal associations with clearly defined objectives. Some of the implications can readily be seen.

From Theory to Practice

SINCE Melin's (*11*) observations that mycorrhizal fungi promoted growth of pine and spruce in drained peat bogs, soils in many parts of the world have been found devoid of fungi that form ectotrophic mycorrhizae. Attemtps to establish trees that normally possess ectotrophic mycorrhizae in some of those areas have failed time and time again. Some dramatic results have been recorded from introduction of mycorrhizal fungi in Australia, parts of Africa, the Russian Steppes, the Caribbean Islands, and in our own midwestern prairies. The fungi usually were introduced into nursery beds as a mixture of organisms in soil from forests or plantations. As mycorrhizal fungi became established on the root systems and the seedlings outplanted, rapid growth responses were very often apparent. One of the

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most recent examples of this has been in Puerto Rico. For approximately 20 years seeds were imported in attempts to establish pine on the island. Twenty-six species and hybrids were introduced. The seedlings, planted in nursery beds, grew to a height of 3 to 12 inches, became chlorotic, and then showed symptoms of extreme phosphorus deficiency evidenced by purple coloration. At this stage they stagnated and died. These studies drew occasional speculation that introduction of appropriate mycorrhizal fungi might permit successful establishment of pine in Puerto Rico.

In 1955, the Forest Service introduced soil inoculum from a stand of pines growing in the southeastern United States to an experimental plot of slash pine seedlings high in the Puerto Rican mountains. Certain trees were inoculated; others were left uninoculated. Within 3 years the effects were dramatic. Uninoculated plants were not more than 12 inches tall and had just a small tuft of needles at their tips. In contrast, inoculated plants had reached heights of up to 8 feet, and were thrifty and fully needled. Thereafter, all seedlings planted on the island were inoculated in a planned program. The Honduras strain of Caribbean pine now grows so successfully that it is not uncommon for the tree to grow 10 feet in one year. Puerto Rico has provided a unique opportunity to study the comparative effects of inoculation with individual species of fungi. This study is now in progress and is supported by the USDA Forest Service and the National Science Foundation. Soon it will be impossible to perform such a study in Puerto Rico because soil throughout the island will become inoculated through spores produced from fruiting bodies of the introduced mycorrhizal fungi.

Nitrogen Sources

AS we have seen, the mineral nutrition of trees is very closely tied to mycorrhizal associations. Since mycorrhizae are most effective in soils containing low concentrations of available nutrients, the question of nitrogen sources has been frequently attributed to possible nitrogen fixation by mycorrhizal fungi. Thus far, no results positively indicate nitrogen fixation by mycorrhizal fungi. Yet, trees can flourish on substrates very poor in available nitrogen. One of the very recent examples of this was reported by Schramm (*17*) on anthracite coal dumps in Pennsylvania. These black wastes are practically devoid of nitrogen, and it has been extremely difficult for vegetation to colonize these areas. The most successful colonization, however, has been by tree species that develop ectotrophic mycorrhizae and a few legumes that have nitrogen-fixing bacteria associated with their roots. Schramm believes that sufficient atmospheric nitrogen reaches the substrate via precipitation to support tree growth. The nitrogen then is absorbed and translocated into root tissues through hyphae of ectotrophic mycorrhizae. This undoubtedly is a source of nitrogen for the plants.

In Australia, however, Richards (*16*) has been working with nitrogen accretion in coniferous forests. This hypotheses indicate the possibility of nitrogen fixation by free-living organisms as large contributors in nitrogen nutrition. One wonders, then, whether some of the nitrogen-fixing bacteria might not be intimately associated with mycorrhizae and thereby contribute to the nutrition of the mycorrhizal fungi and in turn the host plants. The anthracite refuse dumps are an excellent area for those wishing to experiment on ectotrophic mycorrihizae without the complications of many other organisms.

Protection Against Root Pathogens

IN yet another area of investigation, the question has often arisen whether or not ectotrophic mycorrhizal fungi protect roots of trees from invasions by pathogenic organisms. For many years workers have suspected that the dense fungus mantle could be a protective barrier. This was a logical supposition. Mycoparasitic fungi are known to attack hyphae of some fungi. Although sporophores sometimes are parasitized, no fungi are known to parasitize the hyphae of mycorrhizal fungi. Following Zak's (*20*) hypothesis, Marx (*10*), of the Forest Service, recently has shown that ectotrophic mycorrhizal fungi in association with pine can be barriers to invasions of pine root tissues by *Phytophthora cinnamomi*. The mechanical barrier of the mycorrhizal fungus is not subject to attack by *Phytophthora*. Several investigators, including Marx, have found that certain species of mycorrhizal fungi are capable of producing antibiotics that can restrict the growth of some other fungi, including pathogens. However, in all of these cases the importance of antibiosis in controlling plant diseases

is still largely unsolved. The extent to which mycorrhizal fungi are involved in preventing root diseases could be significant. We have data, however, that indicate that vigorous nonmycorrhizal roots in soil are not necessarily attacked by pathogens. Unfavorable soil moisture, aeration, or other edaphic factors that weaken the root or favor invasions by pathogens rather than mycorrhizal fungi need to be further defined.

THE CURRENT RESEARCH EFFORT

THE research effort on mycorrhizae is scattered throughout the world. In Europe—Sweden, Finland, and England have recently been the most active centers for mycorrhizal studies. Certainly Professor Melin in Uppsala, Sweden, deserves special recognition for his 40 years of outstanding continuous work and leadership in mycorrhizal research.

In the United States most research is concentrated in laboratories of the USDA Forest Service. One or two professional scientists at each of five locations are doing mycorrhizae research. Only at our laboratory in Beltsville, Md., is there a full-time study devoted to these phenomena. Elsewhere an occasional graduate student is attempting a study on mycorrhizae. Unfortunately, only a few university staff members are researching the subject; therefore, students must depend heavily upon literature, correspondence, or other contacts with mycorrhiza research workers to aid them.

A very rewarding and unifying research effort has developed through U.S. Public Law 480 programs in foreign countries. Specialists in Finland, India, and Poland have been working on fundamental ecological and physiological studies on ectotrophic mycorrhizae. The widespread geographic distribution of these studies, coupled with others in universities and governmental laboratories here and abroad, are providing valuable new data. These data are defining many of the organisms associated in mycorrhizae, their physiological and morphological peculiarities or similarities, knowledge on habitats, and other factors. We are fortunate that the scientists working on these projects are very capable. We hope it will be possible to have at least one study in an arid country where unique conditions of temperature and moisture exist.

The Mycorrhiza Working Group of the International Union of Forestry Research Organizations, comprised of 15 members from several countries, is filling a special need for mycorrhiza researchers. This is the only international coordinating group for mycorrhizal research. It meets formally every 6 years at the general meetings of the parent organization. The next meeting will be in Munich, Germany, in September 1967, where scientific papers will be presented by members and other mycorrhiza specialists from many countries.

Mycorrhizal associations in natural habitats are more the rule than the exception. It would be encouraging if more well-trained young scientists would accept the challenge of unraveling the complexities of mycorrhizae. However, most textbooks on general botany and plant physiology only lightly mention the phenomenon. To intelligently discuss nutrient uptake by pine roots, for example, one should supplement the traditional theories on absorption through root hairs since root hair development is suppressed on the small feeder roots of pine. This requires an understanding of recent developments on nutrition through mycorrhizal systems. More and more, a realistic approach to the subject is encountered in scientific journals. Textbooks would indeed be of more help in providing backgrounds for students if they included current information on nutrient uptake by mycorrhizae and not just repeat a few, usually only partially accurate, wornout phrases on the subject.

Because mycorrhizal associations are complex, the organisms fastidious at best and sometimes impossible to grow, and because research results usually develop very slowly—few workers have become proficient in the field. Yet we are now in an era of intensive studies in soil biology. Any and all reasonable and thorough approaches to the mycorrhizal problems can only strengthen our knowledge on the subject where so many important gaps still remain.

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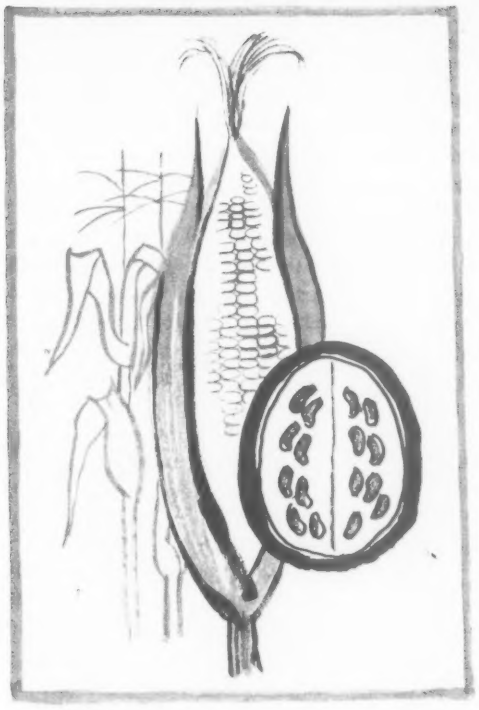

Some Answers and Questions ON HETEROSIS

IGOR V. SARKISSIAN

OPERATIONAL descriptions of heterosis have not yet been advanced. That is, it has not been possible to define heterosis in terms of actual operations or experimental treatments that may have been applied to heterotic and non-heterotic individuals to test the behavior of the hybrid and the control of such behavior. Primarily for this reason, our knowledge of the intimate mechanism(s) of heterosis has not flourished in spite of the fact that we have been aware of heterosis since the time of Koelreuter and Darwin in the 18th and 19th centuries.

Empirical descriptions of heterosis in various organisms have been made many times and are based generally on measurement of factors such as yield of grain, leaf area, height, and dry matter accumulation in plants. In animals and fowl, measurements of rate of gain of body weight, litter size, butterfat content of milk have revealed heterotic behavior of single cross hybrids. In all cases the conclusion was reached that a hybrid exhibits heterosis according to a given measurable attribute.

That heterosis is a genetic phenomenon cannot be doubted. In fact, one can be somewhat specific in describing the genetic makeup necessary for heterosis by stating that heterosis is associated with heterozygosity. Statements that go beyond this point in attempting to explain further the genetic aspects of heterosis are rather general and vague. Dominance, masking of harmful recessive genes in the heterozygote, epistasis, overdominance (*2*),[1] adaptive superiority of the hybrid (*6*), or "physio-

This work is supported in part by National Science Foundation Grant GB-5518.

Published as scientific paper No. 944 with the approval of the Director of West Virginia Agricultural Experiment Station.

[1] Italic numbers in parentheses refer to Literature Cited, p. 25.

logically active" genes, controlling reactions responsible for heterotic expression (*4*)—all of these unfortunately are circular definitions stating in essence that a given organism exhibits heterosis because it is superior (or is not inferior). Hence such definitions do little to advance our understanding of how heterosis starts and how it is accomplished. In all probability, such hypothetical models could account for some aspect of heterosis. However, it would not be incorrect to state that the genetic mechanisms responsible for heterosis have not been demonstrated operationally, although many workers have given a great deal of attention to this problem. Admittedly, a clear explanation of genetic mechanism(s) responsible for heterotic expression is not as yet possible. Much work needs to be done to describe precisely the genetic constitution required of heterotic individuals. The success of such endeavors depends largely on understanding the nature of heterotic growth and development.

We have been studying heterosis with an alternative approach, having decided at the outset that learning the specific genetic requirements for heterotic expression was a formidable and, at present, insurmountable task. My students and I reasoned that a more fruitful approach would be to study mechanisms and reactions responsible for growth and development in general, since heterosis, for the most part, is a manifestation of a superior growth rate of a hybrid over its parents (*1*). We felt, however, that studying the reactions themselves would not be sufficient. Rather, attention should be focused on (a) those metabolic steps which would be of primary importance in an actively growing system, and (b) agents controlling these steps or reactions. This approach, presumably, would be a big step in the direction of the answer to the question, "How is heterosis accomplished?"

Biochemistry and Physiology of Heterosis

INFORMATION on the biochemistry and physiology of heterosis is scant—probably for two reasons. First, most research has been directed toward explaining the genetic makeup required for heterosis. Second, most documented observations were made on the adult individual. In regard to the second reason, Whaley (*14*) noted that heterosis in plants is due in part to a period of rapid initial growth. Obviously, physiological and biochemical studies of heterosis would have to be done on very young individuals. A case in point is the work of Moss who observed that maize hybrids show no superiority to their parents in photosynthesis (*8*). Significantly, the plants used were past the flowering stage. However, a very marked superiority in photosynthetic CO_2 fixation by maize hybrids was observed in our laboratory when young seedlings were used (*10*). It was also reported that young hybrids of barley yielded a cell-free preparation which showed heterosis in terms of photosynthetic carboxylation (*9*).

In continuing studies of heterotic physiology of maize, observations of germination patterns of heterotic and nonheterotic maize hybrids and their parents revealed that only the heterotic hybrid increased in reducing sugar content and decreased sharply in sucrose content; the heterotic hybrid exhibited an earlier onset of radicle emergence. In respiration (oxygen uptake) the heterotic hybrid exceeded its parents and the nonheterotic hybrid, indicating a faster rate of metabolism (*11*). These results suggested that a gibberellin or a gibberellin-like compound was involved in hybrid germination. Later studies verified this hypothesis (*12*).

Thus, several avenues of attack on hybrid biochemistry became available: (1) Although the observation that gibberellin is involved in hybrid growth is an interesting observation, it is a relatively static one. More pertinent information would describe the mechanism of synthesis and action of the gibberellin in the cells of the hybrid. (2) Study of photosynthetic activity of hybrids and controls of such activity would be of interest. (3) Since it was observed that hybrids respired at a higher rate than did the inbreds, study of the respiratory metabolism of hybrids and their parents would be of interest, especially because this would involve studies of the Krebs cycle—the chief energy producing metabolic pathway.

Because a clear understanding of synthesis and the mechanism of action of gibberellin presented as difficult a problem as the entire problem of heterosis, we decided not to attack the problem from that particular vantage point. Since respiration starts very early in the growth of maize (3–4 hours after imbibition of water by seeds) and since photosynthetic activity is not apparent until leaves emerge (5–6 days), we decided to study respiratory metabolism.

Respiration—consumption of oxygen—is associated with subcellular particles, the mitochondria. Mitochondria are also sites for synthesis of ATP (adenosine triphosphate) which is chemically coupled with oxygen uptake. Thus, it is immediately apparent that activities of mitochondria—oxygen uptake and synthesis of ATP—could reflect heterosis. (ATP synthesis can be measured by determining the amount of phosphate utilized by mitochondria.) This would be especially true if heterosis were manifested by superior rate of growth since such growth would depend in part on an abundant supply of usable chemical energy.

Since it is the mitochondrion, the so-called "powerhouse" of the cell, that supplies this energy in the form of ATP, we extracted mitochondria from seedlings of different lines of maize and measured their activity in a standard Warburg respirometer. It was very encouraging to note that mitochondria from hybrids showed distinct heterosis with respect to oxygen uptake and phosphorylation (*7*). Of utmost importance was the observation that hybrids which are nonheterotic, as measured by rate of growth of the seedlings, did not exhibit mitochondrial heterosis. Thus we learned that morphological heterosis—rate of germination and growth of the seedling—is correlated with mitochondrial respiration and production of available energy. Essentially similar results were obtained by Hanson and his coworkers (*5*).

Mitochondrial Origin

THE possibility that one mechanism of heterosis is mitochondria-oriented raised many more questions than it answered. In broad terms, what is the nature of genetic transmission of mitochondrial specificity from parent to offspring? Are mitochondria of hybrids different from those of parents of that hybrid? Are mitochondria from a heterotic hybrid different from those of the parents of that hybrid while the mitochondria of the nonheterotic hybrid are not different from those of its parents?

Several schemes for mitochondrial origin have been suggested. We chose to consider the possibility that mitochondria arise from existing mitochondria and that they are then passed on gametically to the hybrid. We reasoned that the resulting mixture of parental mitochondria could show superior behavior if some form of mitochondrial complementation were to occur in the cells of the heterotic hybrid. A test of this hypothesis would be to make mixtures of mitochondria of the parents and to compare the behavior of the mixture with that of the hybrid. We were aware that such a test could only establish complementation of parental mitochondria; it would not resolve the question of mitochondrial inheritance.

Mixtures were made from parents which (a) produce a heterotic hybrid, and (b) produce a nonheterotic one. Activity of these mixtures were measured in a respirometer and we observed that mixtures of mitochondria of parents of heterotic hybrids do exhibit complementation; that is, the activity approaches that of mitochondria of the hybrid. Mixtures of mitochondria of parents of nonheterotic hybrids do not exhibit such complementation (*7*). These observations led us to two conclusions. Since complementation by parental mitochondria reflects heterosis, such complementation may serve as an operational means of study of heterosis. Of course, the phenomenon of complementation—the mechanism whereby mitochondria exhibit complementary activity—is of great interest and is being studied intensively in our laboratory. The second conclusion was that complementation was a reflection of mitochondrial differences and that the hybrid may well be polymorphic with respect to its mitochondria. This indicated that a qualitative study of mitochondria was essential.

We extracted mitochondria from very young seedlings of maize (12 hours after placing the seeds in moisture) and placed a layer of such mitochondria on a linear sucrose density gradient in ultracentrifuge tubes. The tubes were centrifuged for 20 minutes at 25,000 r.p.m. Mitochondrial sedimentation following the centrifugation is shown in figure 1 (*13*). The mitochondria sedimented in the bottom portion of the tubes, material in the upper portion being mostly vesicular. The data demonstrate that maize is polymorphic with regard to its mitochondria; that is, that Ohio 45 has one type, Wf 9 has two, and Wf 9/Oh 45 has three types of mitochondria. Specifically, the three lines have one type in common (the lowest band); Wf 9 and the hybrid have two types in common—

the upper and the lowest band; the hybrid, in addition to the two types present in the parents, has a third type, the middle band.

Activity of these mitochondrial types in terms of rate of cytochrome C oxidase is summarized in table 1. In comparing the activities of the three lines, it is evident that the hybrid is heterotic because it possesses the "middle" type of mitochondria which contributes approximately 30 percent of the total activity of the hybrid. Without these mitochondria, the hybrid would exceed Wf 9 by only 13 percent.

The precise nature of the "hybrid" mitochondria is not known. However, mitochondrial polymorphism could be expected to be adaptive and could contribute to increased homeostasis of mitochondrial constitution. Such increased homeostasis could provide a physiological, biochemical basis for heterozygote superiority (*3*).

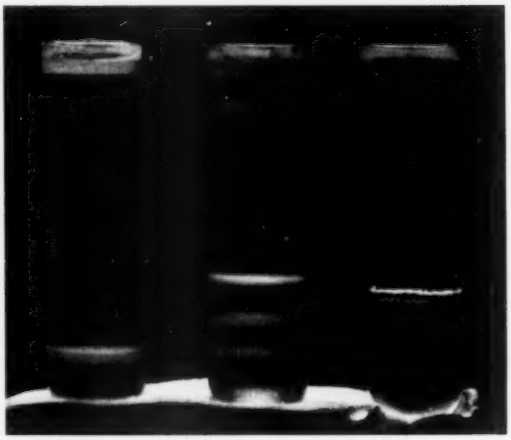

FIGURE 1.—Sedimentation of maize mitochondria in a linear sucrose density gradient. From left to right, mitochondria from scutella of Ohio 45, Wf 9/Oh 45, and Wf 9. Material near the top of tubes is vesicular; mitochondria sediment in lower half of the tube.

TABLE 1.—*Cytochrome C oxidase activity of mitochondria from Ohio 45, Wf 9, and Wf 9/Ohio 45 maize*

Type [1]	Ohio 45	Wf 9	Wf 9/Ohio 45
	Rate of cytochrome oxidation[2]		
Upper		0. 380	0. 464
Middle			. 337
Lower	0. 303	. 374	. 375
Total activity	. 303	. 754	1. 176

[1] Type refers to position in the centrifuge tube.
[2] Increase in optical density at 550 mμ/60 sec/mg. protein.

Status of Our Knowledge

IN summary, what have we learned about heterosis? It would be fair to say that we have learned that heterosis can be observed with mitochondria. We have learned that operational studies of heterosis are possible since parental mitochondria exhibit complementation when artificially mixed (*in vitro* heterosis?). We have learned that maize is polymorphic with regard to its mitochondria and that the hybrid, in addition to parental-type mitochondria, is characterized by a mitochondrial type not present in the parents. Where do we go from here?

One problem which needs to be studied intensively deals with the mechanism(s) of complementation. While the hybrid has the third type of mitochondria, which adds significantly to its total mitochondrial activity, research in progress reveals that the parental-type mitochondria of the hybrid also exhibit complementation when mixed together.

Another problem which must be solved is that of mitochondrial inheritance. Hybrids have parental-type mitochondria which presumably are

passed on gametically to the hybrid. If this is true, how do the "hybrid" mitochondria arise? The same upper and lower types of mitochondria are present in the hybrid and in Wf 9 (fig. 1). Yet Wf 9 does not have the middle band. Does this mean that in the hybrid these two types interact (obviously they do so in terms of activity since they show complementation), and does this interaction lead to a third type, the "hybrid" mitochondria, the formation of which is controlled by the hybrid nucleus? This would explain why Wf 9 does not have the intermediate type of mitochondria since Wf 9 and the hybrid have different nuclear composition. Since complementation is a reflection of heterosis, can it be used as an index of combining ability of two lines? If this were possible, much time and effort could be saved in large-scale breeding operations.

These, then, in brief form, are some of the answers and questions emerging from our work. Clearly, the questions outweigh the answers in number and significance and thus the course is quite clear—continuation of these studies with undiluted diligence and increased intensity.

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Keeping Rain Wet

A small rocket is blasted into the atmosphere from a collective farm in the Soviet Ukraine. Fifteen hundred miles to the southwest an Italian fruit grower launches his own miniature missile. A political crisis? No. This picture might fit farmers of the future at war with a common enemy—hail. They hope to use rockets to seed the clouds with silver iodide, causing moisture to condense and fall as rain before conditions develop which could turn the droplets into hail.

The techniques being advanced to eliminate or modify hailstorms in Europe have made American scientists aware of how little is known about hail activity in the United States. Before methods can be developed to deter this costly and damaging whim of nature, studies must be made to determine the norms of hail damage over a period of years within specified regions. Also the properties of the hailstones themselves must be analyzed so that techniques for combating the storms can be accurately evaluated.

Climatologist Stanley A. Changnon of the State Water Survey at the University of Illinois has been given a National Science Foundation grant to conduct such a study in East Central Illinois, a region representative of most of the Midwest. The study will be conducted over a 2-year period within a hundred miles of Champaign-Urbana.

All data recorded on local hailstorms since 1900 will be gathered and analyzed; trends will then be tabulated and projected into the future. Changnon also hopes to prevail upon some 2,000 farmers in the area to keep regular, detailed accounts of hail activity on their property during the 6-month hail season starting next April. They will be asked to record not only the date and time of the storms, but also the size, frequency, and angle of fall of the hailstones themselves. Their findings will then be compared with the information obtained from radar echos, in order to evaluate the reliability of radar as a prognosticator of hail-bearing storms.

To supplement this effort, plans are being developed for a mechanical apparatus which would automatically record the force of impact, size, angle, and concentration of hailstorms in sparsely populated areas. Such a device, now being designed in cooperation with the Department of General Engineering, would be equipped with an electrical timing mechanism which would indicate the date and duration of the storm. One model now being considered is simply a 14-inch polystyrene mushroom. The depth, concentration, and position of indentations resulting from hailstones falling on the head and stem of this apparatus would indicate a number of properties of the stones. If all goes as planned, the study should indicate the best means of assessing results from future hail modification programs.

From: *Engineering Outlook*, 7:10, 1966
University of Illinois, Urbana

INDEX AVAILABLE

Copies of an index of *Agricultural Science Review* are now available and will be mailed to anyone upon request. In mimeographed form, this index covers the first four volumes of *Review*. Alphabetical listings are given for both author and title of article. A printed index covering the first five volumes will be published in the Fourth Quarter 1967 issue.

New Techniques in FRUIT VOLATILES RESEARCH

R. A. FLATH

FRUIT aroma is one of the major features, along with appearance, in determining the appeal of that fruit to consumers. During consumption, this aroma is again of major importance in contributing to the overall flavor character of the fruit, as the volatile compounds which constitute the aroma are swept into the olfactory region of the nose. (By food flavor we mean the combined impression of that food on both the olfactory and taste senses.) Because of the tremendous sensitivity of the nose, only very small quantities of volatiles need be evolved by a fruit or fruit product. But without these small quantities, the flavor of the food would be dull indeed.

New fruit varieties are developed for a number of reasons: A geneticist may be seeking better disease resistance, higher yield, greater tolerance to poor soil and climate, or better texture for processing. But he cannot completely ignore the flavor of his product, for unless it displays a favorable composition and concentration of volatiles the new variety will be poorly received by consumers.

Change in volatiles composition is a problem for the fruit processor who seeks to maintain a consistent aroma character in his fruit product, whether it be dried fruit, preserves, juice concentrate, or canned fruit. During processing, the aroma make-up of fresh fruit may be considerably altered by partial loss of volatiles, particularly if the fruit is cooked or concentrated. To further complicate matters, undesirable and desirable volatile artifacts may be produced during processing. For example, hexanal and 2-hexanal are not present in any appreciable amounts in the vapor given off by whole fresh Delicious or Gravenstein apples. However, when the cellular tissue of the apple is broken during peeling and slicing, enzymatic action is initiated which causes large increases in the concentrations of the two aldehydes (*1*).[1] These compounds, to which the human nose is rather sensitive, change the character of the fresh apple aroma. Similarly, fruits stored before sale or processing are subject to an

[1] Italic numbers in parentheses refer to Literature Cited, p. 32.

alteration in aroma makeup as their metabolism continues. Some kinds of fruit tend to absorb extraneous volatile material from their environment, whether these compounds are from the construction materials of a storage warehouse, from other nearby foodstuffs, or from the packaging into which the fruit materials are placed for shipment and sale.

The geneticist, the farmer, and fruit processor all need to make their product as appetizing as possible to the public. Aroma and flavor are very subtle properties, varying not only from variety to variety, but also with the degree of ripeness of a given variety, and with the treatment received by the fruit after harvesting.

For several reasons, reliable evaluation of a fruit or fruit product aroma is very difficult. In the first place, the aroma is composed of numerous compounds in a great range of concentrations. Moreover, the evaluation attempt usually consists of the very subjective judgments of a single person or small group of individuals. In order to increase the reliability of such evaluation, many larger processing concerns have found it necessary to establish and train flavor panels. Although the judgments of a sufficiently large and well-trained panel are reliable and very useful, panel work is time-consuming. If a large panel is used, the evaluations become quite expensive as well, so this approach is not feasible for day-to-day use in routine checks of fruit ripening, storage, or processing. Panel evaluations are still subjective, of course, and deal only with the overall impact of an aroma or flavor upon the senses. Therefore, this method provides little if any information about the identities and relative concentrations of the volatile compounds which constitute a fruit or fruit product aroma.

Considerable impetus has been given to fruit volatiles investigations by the advent of gas-liquid chromatography (GLC). This analytical technique, when properly applied, is admirably suited times alone for identifying eluted compounds, With sufficiently high resolution columns and sensitive detectors, very complex mixtures can be separated into their components in reasonably short periods of time. Such analyses can be made quantitative, as well, with the proper ancillary equipment. However, GLC analysis is only a separation and detection technique; it neither identifies nor does it provide an evaluation of an individual compound's impact upon a person's sense of smell. A number of workers have attempted to use retention times alone for identifying eluted compounds, but this is a very uncertain method in natural products work. Fruit volatiles mixtures commonly contain tens to hundreds of components, and very few, if any, GLC columns provide sufficient resolution for separation of all of these compounds. What high resolution gas-liquid chromatography has done is to make the flavor chemist aware of the complexity of volatile mixtures from fruit.

CONCENTRATION APPROACHES

BECAUSE volatiles comprise such a small proportion of most fruits, some concentration step is usually necessary to provide sufficient starting material for the chemist. Ideally, this concentration method should separate the volatiles from the fruit material without changing the relative concentrations of the compounds, and without producing artifacts or introducing extraneous compounds. One or another of these requirements is compromised in most of the methods commonly used.

Solvent extraction of fruit essence or acqueous aroma concentrate has been used by many workers. It is probably the simplest approach when appreciable quantities (grams to hundreds of grams) of concentrate are required for subsequent separation and isolation schemes. There are several drawbacks to this concentration method, however. Essence is prepared by a partial flash evaporation or distillation of fruit juice under vacuum; the condensed material, which is considerably enriched in organic volatiles, constitutes the essence. The extent to which the organic content of the essence represents the aroma composition of fresh fruit depends both upon the operating parameters of the essence preparation equipment, and upon the quality of the juice feedstock. Even if the prepared essence is of very good quality, the solvent extraction sequence is a potential source of trouble. The solvent may be selective, preferentially extracting less polar volatiles from the aqueous essence. It must be of high purity, and preferably be a single compound, for any higher-boiling impurities will be concentrated along with the desired volatiles on removal of solvent. If low-boiling fruit volatiles are of interest, the solvent must be very low-boiling, and special care is required during removal of solvent from the extract to minimize loss of these compounds.

Activated carbon adsorption beds and refrigerated traps have found increasing favor with investigators as techniques (such as GLC) for analyzing small quantities of concentrates have been improved. The traps are used to scrub volatiles from the atmosphere around stored fruit material. If the trapping action is complete, material collected should be representative of the aroma given off by the fruit. Cold traps are less likely to cause decomposition or artifact formation than activated carbon, but their use requires a careful choice of flow rates, system design, and trap temperatures to avoid partial loss of volatiles. The scrubbing action of activated carbon is very thorough, but desorption of the volatiles, once trapped, can be quite difficult, since high temperatures and high vacuum are usually employed to transfer components from the carbon to cold traps. Because of the high water content of fruits, techniques employing cold traps suffer from the problem of separating organic components, including water-soluble compounds, from the water which is also collected.

Both isolation techniques are useful for qualitative work; solvent extraction makes available fairly large quantities of concentrate, while trapping methods—especially cold trapping—provide a concentrate more likely to be representative of the fruit sample, although the quantity obtained is usually fairly small. A choice between these two approaches depends in large part upon the separation and identification methods available to the investigator.

SEPARATION AND IDENTIFICATION

MOST investigations before the late 1950's typically involved laborious fractional distillation of aroma concentrates, followed by chemical examination of each fraction, with preparation of solid derivatives for component identification. However, as workers recognized the great potential of GLC for rapid separation of complex mixtures, a considerable expansion of fruit volatiles investigations began. Researchers now have a tool which enables them to see the complexity of aroma mixtures, and which further permits them to follow changes in volatiles makeup as fruit ripens or is processed. By taking advantage of the high sensitivity of flame ionization detectors, these investigators can completely bypass any preliminary concentration step before GLC separation. A sample of the atmosphere above a fruit or fruit product, when injected into a gas chromatograph, affords a chromatogram representative of the aroma makeup of the fruit sample (*6*).

A major limitation of gas-liquid chromatography was immediately recognized; it is only an often-imperfect separation technique—not a means of identification. Various approaches have been taken to circumvent this latter problem. By running a GLC analysis of a mixture, treating the mixture with some reagent, then rerunning the chromatogram, some indication of kinds of functional groups present in the original mixture may often be obtained (*5*). In combination with retention time measurements on several different stationary phases, this procedure has been applied with some success to the identification of fairly simple compounds. A much more satisfactory procedure involves the use of preparative GLC columns to permit isolation of individual components for subsequent spectral and chemical identification. This is a rather tedious method, particularly with complex mixtures, but is one of the more reliable. Because of the very small quantities of pure components isolated (often submilligram), special techniques have been developed to obtain good infrared and nuclear magnetic resonance spectra.

A very useful, but expensive, identification method is the combination of a fast-scan mass spectrometer and a gas chromatograph (*4*). Since its first preliminary description in 1959, combination MS–GLC has been widely applied in food

volatiles research. It is particularly useful when a high-resolution capillary column is employed, for peak overlap is then greatly reduced, simplifying mass spectral interpretations. Mass spectra obtained under fast-scanning conditions do not always permit unambiguous component identifications, particularly if these components are minor, so MS identifications are usually checked by coinjection of an authentic sample of the identified component along with the entire mixture. A flame ionization detector is used to follow this analysis.

Direct vapor injection into a MS–GLC combination is a logical extension of this separation-identification method. All opportunities for artifact formation during aroma concentration are then eliminated, so that the analysis provides a reliable picture of the fruit sample's aroma makeup. One group has recently reported using this technique with tea and coffee aromas (*3*), but no reports of its application to fruit volatiles have as yet appeared. Subject to the limitations of mass spectral identification, this will undoubtedly be an extremely useful way to rapidly survey the makeup of many different fruit and fruit product aromas.

ORGANOLEPTIC EVALUATION

COMPONENT identification is only half of the problem facing a flavor chemist. He also needs to know which of the many identified volatiles given off by a fruit are significant contributors to the overall aroma impression. This requires the use of a trained judging panel, to which carefully purified compounds and mixtures of compounds are submitted for aroma comparison with the fruit or fruit product. The threshold, or detection level, of each component should also be determined by the panel. Because of the great range of sensitivities of the human nose to different compounds, the threshold of the volatile must be considered, along with its actual concentration, in evaluating its contribution to fruit aroma.

Several workers have found it convenient to have individual judges sample the GLC column effluent stream after its passage through a nondestructive thermal conductivity detector, even before any identifications are undertaken. By correlating the judges' aroma descriptions with the recorded gas chromatogram, promising components may then be pinpointed for subsequent identification. The use of a large-bore capillary column provides the resolution necessary to make this approach practicable with complex mixtures (*2*).

At the present time, many groups throughout the world are carrying out research aimed at identifying components of fruit (and other food) volatiles. This impetus is due in large part to the recent advances in instrumentation mentioned above. Unfortunately, attempts to correlate the presence and concentrations of these many constituents with the overall aroma impression of a fruit material are not proceeding at nearly the identification rate.

There are a number of reasons for this present situation. A major problem is the complexity of the instrumentation used—namely, the olfactory system and brain of the judge. This apparatus is not likely to be changed, so any improvement must occur in the mechanics of the judging process, which is quite time-consuming at present. Also, samples submitted to a panel must first be scrupulously purified; because of threshold variations, the human nose may be more sensitive to an impurity than to the major component. GLC purity is a necessary, but not always sufficient requirement, for very minor impurities may still modify the aroma of the sample. Similarly, odors of the environment may have a significant effect upon a panel's

ability to function in a consistent manner. Unfortunately, organoleptic evaluation is not likely to undergo the sort of dramatic improvement that has occurred in recent years in identification techniques.

This area—organoleptic research—requires a greater number of investigators, but equally important, it requires painstaking care in all phases of the evaluation, from purification of samples to evaluation of data.

At present much of the published organoleptic work originates in governmental and academic laboratories both in the United States and abroad. For example, investigators in the U.S. Department of Agriculture have found such research to be indispensible in their efforts to pinpoint those compounds which appear to be important contributors to the aroma and flavor of certain foods. The Food and Drug Administration is involved in similar work, but their major emphasis is on components which appear at the onset of spoilage, or upon contamination of food. Many of the academic institutions with strong food science-oriented departments also include within these departments some people doing both identification and organoleptic evaluation work. Undoubtedly, the larger food companies and flavor houses are conducting such studies too, but most of the resultant information is in the nature of trade secrets, so it is not available to the food industry as a whole.

APPLICATION OF RESULTS

ALTHOUGH gathering analytical and organoleptic information is challenging and interesting *per se* to the researcher, the results can also be of great value to the fruit industry. The major problem here is one of application. Because of the capital outlay requirements for equipment and the cost of maintaining laboratory personnel, much of the application work in the immediate future is likely to be done by the larger processing companies. As processors are successful in improving the quality of their product, growers should also benefit, because of an increased market for the improved product. Ultimately, of course, consumers also benefit, as more appealing fresh and processed fruit materials reach the market.

Utilization of these data can take many forms. Merely being aware of the nature of important compounds in fruit aroma can be of value to the processing engineer, for he can then avoid conditions in processing streams which would tend to degrade such compounds or cause their loss by volatilization. However, using sensitive GLC equipment in conjunction with accumulated composition and aroma data can benefit the fruit industry in many additional ways. For example, a processor could evaluate the volatiles makeup of his starting material, and then blend different lots or varieties to some standard composition, if desirable.

Gas-liquid chromatography could aid in developing new varieties for processing. Fruit stored after harvest, but before processing or sale as whole fruit, can be periodically checked for volatiles loss or change, which may indicate the beginning of deterioration during storage. Similarly, off-aromas picked up by fruit or fruit products, either from the storage environment or from packaging materials, may be detected and traced to their origin. Changes in aroma composition during processing can be followed and the results used as a means for improving product quality.

Of necessity, the identification of components must usually rely upon retention time data, for more reliable methods require expensive equipment and considerable time. The degree of success with which retention times may be employed in assigning component identities depends in large part upon the quantity of reliable composition data available on that given fruit variety as well as upon the quality and reproducibility of the GLC separation.

Retention comparisons should be made on high-resolution columns—preferably on at least two different stationary phases. For their composition data, workers in the fruit industry must rely largely upon government, academic, and perhaps a few industrial laboratories. The biggest shortcoming of the exclusive use of retention times is that it does not deal effectively with new, previously unidentified components—whether they are artifacts from storage or processing or volatiles from the environment of the fruit. As on-stream devices such as fast-scan infrared instruments become less expensive and more sensitive, this situation will change, but at present the limitation must be recognized. For this reason, a continuous flow of information from government and academic laboratories is necessary to assist the fruit industry in maintaining and improving the quality of their products.

FUTURE DIRECTIONS

IN the next few years we may expect considerable expansion in this field, particularly in compound identification. The mass spectrometer-gas-liquid chromatograph combination is rapidly becoming a common tool in composition investigations, and once the problems associated with direct vapor analysis using MS-capillary GLC are surmounted, the true potential of this powerful technique should be better realized. Computer normalization and analysis of the resultant mass spectra should minimize a significant drawback of MS–GLC to an analytical chemist; namely, digestion of the large quantity of raw data. Because of the small sample requirements of vapor analysis, very useful ripening and spoilage studies will be feasible.

Organoleptic evaluation work will probably fall even farther behind, partly because of the scarcity of qualified people to direct this type of research. This lag is unfortunate, because identification and evaluation are complimentary; with such sensory information, the value of composition data to flavor investigations is considerably enhanced.

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Teamwork in Scientific Research

A. C. HOFFMAN

TEAMWORK in scientific research has become an absolute necessity. Scientists all over the world are beginning to realize that a satisfactory solution for any specific problem lies in the pooling of the knowledge of men from different scientific disciplines.

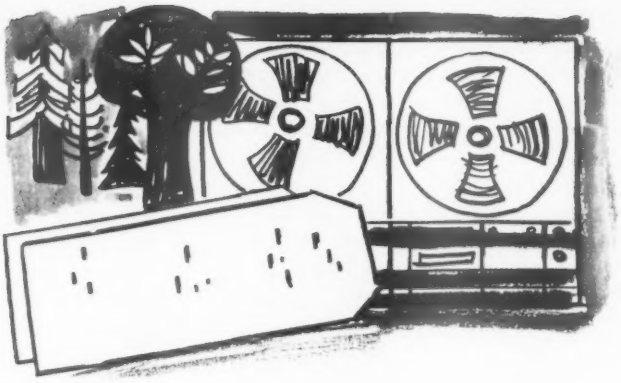
To know mankind, one has to know all varieties of mankind; knowing people means seeing them as people and this means a knowledge of oneself, one's own anatomy, one's own physiology, one's own origin and history, and one's own soul.

To understand and to describe mankind correctly, teamwork and collaboration between anatomists, physiologists, anthropologists, archaeologists, historians, and sociologists seem to be essential.

My plea for teamwork in scientific research is based on the conviction that science should follow an unfaltering search for truth, with a belief in the betterment of humanity through knowledge. This should be the ethical basis of science, and none other.

Today we live in a world with a total population of over 3,000 million, and in this world there are many problems facing humanity, the biggest of which is perhaps the feeding of the peoples of the world. I firmly believe that the salvation of this world lies in the hands of the scientists. A better understanding amongst the scientists of the world and a closer cooperation amongst ourselves may lead to the expulsion of the horrors of war and to the total extermination of the dangers of starvation.

Condensed from "The Necessity of Teamwork in Scientific Research," Presidential Address to the 64th Annual Congress, South African Association for the Advancement of Science, held at Stellenbosch, July 1966. Reprinted, with permission, from *South African Journal of Science*, 62: 12, December 1966.



International Tree Disease Register

GEORGE H. HEPTING

For conceiving and developing INTREDIS, Dr. Hepting received in March 1967 a special merit award in Secretary Freeman's program for outstanding cost reduction achievement.

AN electronic system of literature storage and retrieval for forest pathology has been put into operation by the U.S. Forest Service in cooperation with the Canada Department of Forestry and Rural Development.

Designed for worldwide coverage of the field of tree diseases, the International Tree Disease Register (INTREDIS) offers a much-needed service to researchers working in forest pathology. The project was sponsored by the FAO/IUFRO Symposium on Internationally Dangerous Forest Diseases and Insects, Oxford, 1964.

INTREDIS enables any qualified person to receive a literature list by author, approximate title of article, and reference to an abstract journal carrying the article. Coverage includes all articles likely to be of interest to a tree pathologist which have been abstracted by *Review of Applied Mycology, Forestry Abstracts, Biological Abstracts,* and *Air Pollution Control Association Abstracts.* Abstract citations dating from the early 1940's through 1965 have already been coded; ultimately, those dating back to 1930 will also be included.

INTREDIS can provide a printout within an hour after receipt of a request, and it is inexpensive. A request may be made by subject (for example, root rot or seed diseases), or kind of tree (for example, white oak or Douglas-fir), or causal organism (for example, *Endothia parasitica* or *Pseudomonas syringae*), or place (for example, Mexico or Australia or all of South America), or date, or author. Thus, the computer can search the magnetic tape in one operation for, say, "nematodes in the genus *Xiphinema* attacking white pine roots in Canada," and print out a list of appropriate references, as easily as it can print out the world literature on air pollution effects on trees.

INTREDIS has some novel features that make it feasible, effective, and reasonably inexpensive. First, a professional forest pathologist assigned to INTREDIS selects the articles for coding by going through each issue of the named abstract journals. His knowledge of the discipline helps him determine what to select and how to code his material for the punchcard operator, who punches one or more 80-column cards for each coded reference, the number depending upon the amount of cross-referencing needed.

It is a cardinal point that the selector and coder be a professional forest pathologist. Who else would know to code the title "Dutch elm disease in winged elm" under host plant *Ulmus alata* and cause *Ceratocystis ulmi;* or that the "spike disease of sandal" is to be coded as a virus-caused disease of *Santalum?* Thus, we do not have incidents like the physiologist whose book "The Translocation of Food in Plants" came out under the subject of "belt conveyors."

The second unique feature of INTREDIS is that it is tied directly to the few abstract journals that cover well the field of tree pathology. Since our literature printouts show the volume and page number from these widely used journals, almost any scientist in our field will have ready access to the citations listed on a printout, and he will then get an abstract, as well, for each. By then, he should know if he wishes to see the full article or book.

The coder, using our published INTREDIS codebook, writes out on a worksheet—for each citation he selects—the subject, host plant, causal organism, and country. All of these are in code. Then, using numbers and English letters, he enters the name of the abstracting journal, its volume and page, the year the article was published, the author, and an abbreviated title. A card-punch operator transfers the worksheet information to coded cards; the card information is then put on magnetic tape.

A computer program for INTREDIS was written in a few days and put on tape also. An inexpensive computer system is used, since our operation is a simple scan (sort) and printout, with no computations to perform. At present our tape contains about 13,000 citations. It can be "read" for whatever topic has been sensed for, and a printout provided in about 15 minutes of a cost of about $20 for machine time.

I have been told that many schemes for electronic literature retrieval have failed because the quality of input was too low. In short, too often the selecting and coding was not done by people who really knew the literature in a given field. Other attempts have failed because what worked well for one field proved to be very poor for another. An economist who aided me in setting up INTREDIS said later: "This system works so well for forest pathology—a well-abstracted field with good access terminology—it is a pity I can't use it for my own field."

Some systems try to do too much and end up doing nothing. One system, expensive to subscribe to, gave me extremely little for a year's search because it tried to cover all sciences. As a result, there was little search of our particular source material. Systems based on keywords in a title are cumbersome for what you get. Again they cover too wide a field, and anyone who has scanned some of these lists knows how long it takes to find an article he is interested in. There is another drawback to the keyword-in-title idea. If, for example, you wanted a particular article on air pollution injury to trees, you might have to look under six or more keywords to find the article since the author may have used one of many alternate key terms. He may have chosen to call it "SO_2 injury to trees," or "fume injury to trees," or "smoke injury to trees," or "air pollution injury to trees." With INTREDIS, however, you would get all air pollution articles by one simple sort on subject code 70. A quick scan of the printout would produce the specific article you were looking for.

Finally, once set up, INTREDIS is easy to operate. When I get a request I tell my secretary what scans to make. She delivers the appropriate computer program cards to the computer center, and within a short time the citation printout is made and is on its way to the requester. Although we are still on a "pilot plant" basis, we have already handled requests from many countries and more than 50 individuals. Tapes are now being run both by the Canada Department of Forestry and Rural Development and the Forest Service of the U.S. Department of Agriculture.

Eventually, when our tapes are expanded to cover literature back to 1930, we also hope to be able to turn the operation over to the National Agricultural Library in Washington, D.C. A charge may then be made for the machine time required to handle a request, but it will still be inexpensive. Even now, a 300-item printout on the world literature on *Fomes annosus* root rot—that ordinarily would have taken months to compile—was done by INTREDIS at a machine time cost of $20.

At present the service is offered without cost to United States and Canadian Government scientists and to others who have a pressing need for it. Requests for printouts should be addressed to the author at U.S. Forest Service, P.O. Box 2570, Asheville, N.C. 28802.

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U.S. GOVERNMENT PRINTING OFFICE: 1967 O—258-908

AGRICULTURAL SCIENCE REVIEW is a critical review journal designed to provide a common point of understanding among agricultural scientists by publishing authoritative commentary on the current state of agricultural research. *Review* does not publish primary research reports. Yearly subscription rate is $1.25, domestic; $1.50, foreign. Single copies are 35 cents. Subscription orders should be sent to the Superintendent of Documents, Government Printing Office, Washington, D.C. 20402.